PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2005/010242	25.03.2005	25.03.2004

International Patent Classification (IPC) or both national classification and IPC
C12N9/42, C12N15/62, C12P21/02, C12P21/06

Applicant
GENENCOR INTERNATIONAL, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2005/010242

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2005/010242

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1,2 ,8-10, 12, 13, 26, 28-30, 33-36, 39-47, 53
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-53
Industrial applicability (IA)	Yes:	Claims	1-53
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. General remarks

1.1 Reference is made to the following documents:

D1: WO 97/27306 A (ALKO GROUP LTD; MAENTYLAE, ARJA; PALOHEIMO, MARJA; LANTTO, RAIJA; FAGE) 31 July 1997 (1997-07-31)

D2: WARREN R A ET AL: "A bifunctional exoglucanase-endoglucanase fusion protein" GENE, ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, NL, vol. 61, no. 3, 1987, pages 421-427, XP002342343 ISSN: 0378-1119

D3: BAKER J O ET AL: "A NEW THERMOSTABLE ENDOGLUCANASE, ACIDOTHERMUS CELLULOLYTICUS E1. SYNERGISM WITH TRICHODERMA REESEI CBH I AND COMPARISON TO THERMOMONOSPORA FUSCA E5" APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY, CLIFTON, NJ, US, vol. 45/46, 1994, pages 245-256, XP000672157 ISSN: 0273-2289

D4: WO 98/31821 A (GENENCOR INTERNATIONAL, INC) 23 July 1998 (1998-07-23)

D5: WO 92/01797 A (OY ALKO AB) 6 February 1992 (1992-02-06)

D6: KERÄNEN ET AL: "Production of recombinant proteins in the filamentous fungus Trichoderma reesei" CURRENT OPINION IN BIOTECHNOLOGY, LONDON, GB, vol. 6, no. 6, 1995, pages 534-537, XP002109010 ISSN: 0958-1669

D7: BERGQUIST P ET AL: "Expression of xylanase enzymes from thermophilic microorganisms in fungal hosts" EXTREMOPHILES, SPRINGER VERLAG, TOKYO,, JP, vol. 6, no. 3, June 2002 (2002-06), pages 177-184, XP002327848 ISSN: 1431-0651

D8: DE FARIA F P ET AL: "Expression and processing of a major xylanase (xyn2) from the thermophilic fungus Humicola grisea var. thermoidea in Trichoderma reesei" LETTERS IN APPLIED MICROBIOLOGY, OXFORD, GB, vol. 34, no. 2, 2002, pages 119-123, XP002342344

D9: NYYSOENEN E ET AL: "PROTEIN PRODUCTION BY THE FILAMENTOUS FUNGUS TRICHODERMA REESEI: SECRETION OF ACTIVE ANTIBODY MOLECULES" CANADIAN JOURNAL OF BOTANY / JOURNAL CANADIEN DE BOTANIQUE, NATIONAL RESEARCH COUNCIL, OTTAWA, CA, vol. 70, no. SUPPL 1, January 1995 (1995-01), pages S885-S890, XP000575039 ISSN: 0008-4026

D10: NYYSSONEN E ET AL: "MULTIPLE ROLES OF THE CELLULASE CBHI IN ENHANCING PRODUCTION OF FUSION ANTIBODIES BY THE FILAMENTOUS FUNGUS TRICHODERMA REESEI" CURRENT GENETICS, NEW YORK, NY, US, vol. 28, no. 1, 1995, pages 71-79, XP008039837 ISSN: 0172-8083

2. Novelty (Article 54 EPC)

2.1 Example 21 of document D1 discloses the production of *Termonospora (= Thermobifida) fusca* cellulases in *Trichoderma reesei*.

2.2 In particular (page 63, second and third paragraphs), it discloses a construct comprising the signal sequence and core (= first catalytic domain) and hinge (hinge= linker) domains of a *Trichoderma reesei* cellobiohydrolase CHBI fused to a *Thermonospora fusca* (bacterial) endoglucanase E1, E2, E4 or E5 (= second catalytic domain). This results in the expression of the fusion protein in *Trichoderma reesei*. Said document is considered as novelty-destroying for claims 1, 2, 8-10, 12, 13, 26, 28-30, 33-36, 39-47 and 53.

2.3 Document D2 discloses a fusion between the cex gene of *Cellulomonas fini*, which encodes an exoglucanase, and the cenA gene of the same organism, which encodes an endoglucanase (see abstract and Figure 3). Also disclosed are the expression of the

fusion protein, the isolated fusion protein (see Figure 5), and crude cell extracts containing the fusion protein (see page 424, right-hand column, first lines of the paragraph). Said document is therefore considered as novelty-destroying for claims 1, 10, 33, 35 and 39.

2.4 Claim 53 is directed to a composition comprising the *components* of a (generic) cellulase fusion protein. A mixture containing 20% and 80%, respectively, of purified *Thermonospora fusca* E5 and *Trichoderma reesei* CHB I is disclosed in document D3 (see Figure 1). Since said (purified) components cannot be discriminated from the (recombinant) components of claim 53, the claim is not considered to be novel.

2.5 Therefore, claims 1, 2, 8-10, 12, 13, 26, 28-30, 33-36, 39-47 and 53 are not novel (**Article 54 EPC**).

3. Inventive step (Article 56 EPC)

3.1 Document D1 is considered to represent the closest prior art with respect of the subject-matter of the present application. The objective problem to be solved resides therefore in the provision of *alternative* fusion constructs for the expression of bacterial cellulases in filamentous fungi.

3.2 In fact, fusion constructs comprising such elements as a fungal promoter such as the *T. reesei* cbh1 promoter; the signal sequence, core and hinge regions of *T. reesei* CBHI; an endoprotease cleavage site such as the kexin site; a alternative bacterial or eucaryotic enzymes or proteins to be expressed; a terminator sequence; a selectable marker, in combination with expression in Trichoderma cells, for the expression of proteins and enzymes with high yield, is well documented in the prior art (see Document D1, examples 14-16 and 21; D4, examples 7 and 14-16; D5, examples 14, 17 and 19; and D6-D10). Also, it is known that the catalytic core of cellulases is active even in the absence of the CBD.

3.3 The application of these technical features in the expression of a further enzyme, is not considered to involve an inventive step for the claims that refer to (bacterial)

endoglucanase(s) in general (claims 1-12, 17-36, 39-42, 44-47, 52 and 53.

3.4 With respect to the more specific claims which refer to endoglucanases from *Acidothermus cellolyticus* and *Thermobifida fusca* strains, it is noticed that document D1 does refer to such endoglucanases E1, E2, E4 and E5 from *T. fusca* in Example 21. It follows that claims 13 and 43 that are directed to a fusion protein comprising a catalytic *T. fusca* E5 endoglucanase, are not considered to involve an inventive step.

3.5 Additionally, even without taking document D1 into consideration, the application does not seem to disclose any surprising technical effect which would justify the recognition of a inventive step. Indeed, the only example for which results have been actually demonstrated (Example 3 and Figure 23) only relates to the fusion of exo-cellobiohydrolase cbh1 and the E1 endoglucanase.

3.6 This applies in particular to claims 13, 38, 43, 49 and 50, which do refer to fusion proteins comprising a catalytic domain of a unspecified or a GH74 endoglucanase from *A. cellulolyticus*, which are considered to be obvious for the skilled person and for which no surprising effects are disclosed.

3.7 The only technical effect disclosed in the application is found in Example 3 and corresponding Figure 23. Said example shows that a fusion construct containing the *A. cellulolyticus* E1 endoglucanase catalytic domain (as in claims 13, 15 , 16 and 50) outperforms the parent. This effect, however, is not considered to be surprising, because document D3 does discloses synergism between A. cellulolyticus E1 endoglucanase and *T. reesei* CBH1. Hence, claims 15, 15, 16 and 50 are also not considered to involve an inventive step (**Article 56 EPC**).